VACCINE COMPOSITIONS CONTAINING LIPOSOMES

This application is a continuation, of application Ser. No. 08/308,622, filed Sep. 19,1994 now abandoned, which is a continuation of application Ser. No. 08/154,160, filed Nov. 18, 1993, now abandoned, which is a continuation of application Ser. No. 07/722,862, filed Jun. 28, 1991, now abandoned, which is a continuation-in-part of application Ser. No. 07/546,585, filed Jun. 29, 1990, now abandoned, from which applications priority is claimed pursuant to 35 USC §120.

BACKGROUND OF THE INVENTION

1. Technical Field

This invention relates generally to vaccines and is particularly directed to techniques and compositions for increasing the efficiency of vaccines and generating neutralizing antibodies.

2. Background

Liposomes are spherical vesicles consisting of a lipid bilayer and an enclosed aqueous space that are typically formed of phospholipids in aqueous solutions. By incorporating chemical or biochemical substances in the aqueous phase of the suspension in which the liposomes are formed, liposomes can be obtained that enclose within their interior space various biologically active substances. Liposomes, which typically vary in size from about 25 nm to about 1 μm in diameter depending on how they are produced and the content of their lipid layer, therefore can be used as delivery vessels for various water-soluble substances. Since charged molecules generally do not penetrate lipid bilayers and since large molecules penetrate such layers only slowly, the liposome wall acts to insulate an organism to whom the liposomes have been administered from too rapid an effect by the enclosed material.

Liposomes have previously been used to modulate the immune response in animals by administering the liposomes with an antigen. The immune response can be modulated either for the purpose of inducing immunity in the host against a pathogen or for the purpose of producing antibodies used outside the host. For example, there is an increasing interest in improving and varying the capability of preparing antibodies to a wide variety of determinant sites for use in immunoassays and immunodirected therapy. The unique capability of antibodies to bind to a specific determinant site or chemical structure makes them peculiarly useful in directing drugs, radioisotopes, or markers to a particular site in a host. In addition, the ability of antibodies to distinguish a specific structure from similar structures has resulted in their wide use in diagnosis.

Regardless of whether one wishes monoclonal or polyclonal antibodies for use outside the host or an immune response to protect the host against a pathogen, the initial step is the immunization of a host. As will become clear below, the composition used in immunization is referred to herein as a "vaccine" regardless of whether the composition is used to protect the host or in the production of monoclonal antibodies. Usually one hyperimmunizes the host by repeated injections of the immunogen in a vaccine composition in accordance with a predetermined schedule. Adjuvants are added to potentiate the immune response. Various adjuvants include aluminum and calcium salts, emulsifying adjuvants and bacteria, e.g., mycobacteria and corynebacteria.

When monoclonal antibodies are desired, it is particularly desirable to enhance the immune response to specific ectopic sites. Since the preparation of monoclonal antibodies requires the detection of low population events, any technique which enhances the B-lymphocyte population of interest can prove to be important in the production of monoclonal antibodies. See U.S. Pat. No. 4,565,696 which describes the production of immunogens by conjugation of antigens to liposomes.

As will be described hereafter, the present invention is directed to the preparation of vaccine compositions containing liposomes. The liposomes, and various other components as will be discussed in detail below, act as adjuvants for increasing the immunogenicity of the antigens derived from or otherwise related to pathogenic agents.

Currently, the only adjuvants approved for human use in the United States are aluminum salts (alum). These adjuvants have been useful for some vaccines including hepatitis B, diphtheria, polio, rabies, and influenza, but may not be useful for others, especially if stimulation of cell-mediated immunity is required for protection. Reports indicate that alum failed to improve the effectiveness of both whooping cough and typhoid vaccines and provided only a slight improvement with adenovirus vaccines. Problems with aluminum salts include induction of granulomas at the injection site and lot-to-lot variation of alum preparations.

Complete Freund's Adjuvant (CFA) is a powerful immunostimulatory agent that has been used successfully with many antigens on an experimental basis. CFA is comprised of a mineral oil, an emulsifying agent such as Arlacel A, and killed mycobacteria such as *Mycobacterium tuberculosis*. Aqueous antigen solutions are mixed with these components to create a water-in-oil emulsion. CFA causes severe side effects, however, including pain, abscess formation, and fever, which prevent its use in either human or veterinary vaccines. The side effects are primarily due to the patient's reactions to the mycobacterial component of CFA.

Incomplete Freund's Adjuvant (IFA) is similar to CFA without the bacterial component. While not approved for use in the United States, IFA has been useful for several types of vaccines in other countries. IFA has been used successfully in humans with influenza and polio vaccines and with several animal vaccines including rabies, canine distemper, and hoof-and-mouth disease. Experiments have shown that both the oil and emulsifier used in IFA can cause tumors in mice, indicating that an alternative adjuvant would be a better choice for human use.

Muramyl dipeptide (MDP) represents the minimal unit of the mycobacterial cell wall complex that generates the adjuvant activity observed with CFA (see Ellouz et al. (1974) *Biochem. Biophys. Res. Comm.*, 59: 1317). Many synthetic analogues of MDP have been generated that exhibit a wide range of adjuvant potency and side effects (reviewed in Chedid et al. (1978) *Prog. Allergy*, 25:63). Three analogues that may be especially useful as vaccine adjuvants are threonyl derivatives of MDP (see Byars et al. (1987) *Vaccine*, 5:223), n-butyl derivatives of MDP (see Chedid et al. (1982) *Infect. and Immun.*, 35:417), and lipophilic derivatives of muramyl tripeptide (see Gisler et al. (1981) *Immunomodulations of Microbial Products and Related Synthetic Compounds*, Y. Yamamura and S. Kotani, eds., Excerpta Medica, Amsterdam, p. 167). These compounds effectively stimulate humoral and cell-mediated immunity and exhibit low levels of toxicity.

One promising lipophilic derivative of MDP is N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-[1,2-dipalmitoyl-sn-glycero-3-(hydroxyphosphoryloxy)] ethylamide (MTP-PE). This muramyl peptide has a phos-